(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 December 2004 (16.12.2004)

PCT

(10) International Publication Number
WO 2004/108157 A2

(51) International Patent Classification[7]: A61K 39/395

(21) International Application Number:
PCT/US2004/017049

(22) International Filing Date: 1 June 2004 (01.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/474,603 2 June 2003 (02.06.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERAPEUTIC AND PROPHYLACTIC METHODS FOR NEUROMUSCULAR DISORDERS

(57) Abstract: The disclosure provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering therapeutically effective amounts of a GDF-8 inhibitor and a corticosteroid to a subject susceptible to, or having, a neuromuscular disorder, so as to maintain desirable levels of muscle function.

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THERAPEUTIC AND PROPHYLACTIC METHODS FOR NEUROMUSCULAR DISORDERS

[0001] This application claims priority to United States provisional application No. 60/474,603, filed on June 2, 2003, which is incorporated herein by reference in its entirety.

Field of the Invention

[0002] The present invention relates to the field of clinical pathophysiology, and more particularly to methods for treating neuromuscular disorders, such as muscular dystrophies. The invention also relates to pharmaceutical formulations containing corticosteroids and inhibitors of growth and differentiation.

Background of the Invention

[0003] Muscular dystrophies (MD) are progressive inherited neuromuscular disorders that are characterized by muscle wasting and weakness (Emery (2002) The Lancet, 359:687-695). Many forms of muscular dystrophies are fatal and currently incurable.

[0004] Duchenne muscular dystrophy (DMD) is the most common X-linked neuromuscular disease. The disease is caused by mutations in the DMD gene coding for dystrophin. Alteration or absence of this protein results in abnormal sarcolemmal membrane tearing. An abnormal variation in diameter of muscle fibers (atrophic and hypertrophic fibers) in proximal muscles and ongoing muscle damage are hallmarks of the disease.

Damaged muscle releases the intracellular enzyme creatine kinase (CK). As a result, the serum CK levels in DMD patients are characteristically high (up to 10 times the normal). The pathophysiologic cascade is compounded by tissue inflammation, myofiber necrosis and replacement of muscle with fibrofatty tissue.

[0005] Another allelic variant of the DMD gene causes a milder form of MD known as Becker muscular dystrophy (BMD). BMD is clinically similar to DMD but the onset of symptoms occurs later in life.

[0006] Many pharmacological agents have been tried in MD but none has proved effective in arresting the course of the disease. The current modality of treatment is still in the realm of physical medicine and rehabilitation.

[0007] A number of trials using corticosteroids (e.g., prednisone and/or its derivatives) have demonstrated improvement in individuals with MD, particularly in the short-term. Although the exact mechanism by which corticosteroids alleviate the disease phenotype is unclear, corticosteroids are thought to act by reducing inflammation, suppressing the immune system, improving calcium homeostasis, upregulating expression of compensatory proteins, and increasing myoblast proliferation (Khurana et al. (2003) Nat. Rev. Drug Discovery 2:279-386). However, corticosteroids administered over time can induce muscle atrophy, which primarily affects proximal muscles—the very same muscles that are affected in DMD and BMD. The

corticosteroid-induced muscle and other side effects may limit the long-term effectiveness of corticosteroid therapy.

[0008] GDF-8 is a member of the TGF-β superfamily and functions as a negative regulator of muscle growth. Similarly to other members of the superfamily, GDF-8 is synthesized as a precursor molecule, but prior to secretion, it is cleaved into the N-terminal inhibitory propeptide and C-terminal the active mature GDF-8. Propeptide may remain bound to GDF-8 thereby inhibiting the biological activity of mature GDF-8. Propeptide must dissociate from the complex for GDF-8 to bind to activin type II receptor (ActRIIB). Upon binding, ActRIIB initiates a signaling cascade, ultimately leading to the inhibition of myoblast progression. Antibody-mediated inhibition of GDF-8 *in vivo* has been shown to significantly increase skeletal muscle size in normal adult mice (Whittemore et al. (2003) BBRC, 300:965-971) and to alleviate the dystrophic phenotype in the *mdx* mouse model of DMD (Bogdanovich et al. (2002) Nature, 420(28):418-421).

SUMMARY OF THE INVENTION

[0009] It is one of the objects of the present invention to provide methods and compositions for treating disorders characterized by or associated with a risk of diminution of muscle function. Additional objects of the invention will be set forth in part in the following description, and in part will be understood from the description, or may be learned by practice of the invention.

[0010] The present invention is based, in part, on the discovery and demonstration that, in a mouse model of DMD, treatment by administration of a neutralizing anti-GDF-8 antibody and prednisone is more effective in increasing muscle mass and strength relative to treatment with prednisone alone. The invention is further based, in part, on the discovery and demonstration that administration of anti-GDF-8 antibody with prednisone reduces prednisone-induced muscle atrophy.

[0011] Accordingly, the present invention provides methods for treating neuromuscular disorders in mammals. The disclosed methods include administering to a subject susceptible to or having a neuromuscular disorder therapeutically effective amounts of at least one GDF-8 inhibitor and at least one corticosteroid so as to maintain desirable levels of muscle integrity or function as assessed by, for example, serum concentration of creatine kinase (CK), muscle histology, tissue imaging, activities of daily living, muscle strength and/or mass. The populations treated by the methods of the invention include, but are not limited to, patients having or at risk of developing muscular dystrophy such as, for example, DMD or BMD, and subjects undergoing corticosteroid therapy for these or other disorders.

[0012] The invention further provides methods of treating muscle weakness and methods of treating corticosteroid-induced muscle atrophy. The invention includes methods of treating cardiomyopathy.

[0013] Methods of administration and compositions used in the methods of the inventions are provided. In the disclosed methods, a GDF-8

inhibitor and a corticosteroid are administered concurrently or over alternating overlapping or non-overlapping intervals.

[0014] GDF-8 inhibitors, used in the methods of the present invention, include, but are not limited to, antibodies to GDF-8; antibodies to GDF-8 receptors; soluble GDF-8 receptors and fragments thereof (e.g., ActRIIB fusion polypeptides as described in U.S. Patent Application No. 10/689,677, including soluble ActRIIB receptors in which ActRIIB is joined to the Fc portion of an immunoglobulin); GDF-8 propeptide and modified forms thereof (e.g., as described in WO 02/068650 or U.S. Patent Application No. 10/071,499, including forms in which GDF-8 propeptide is joined to the Fc portion of an immunoglobulin and/or form in which GDF-8 is mutated at an aspartate (asp) residue, e.g., asp-99 in murine GDF-8 propeptide and asp-100 in human GDF-8 propeptide); a small molecule inhibitor of GDF-8; follistatin (e.g., as described in U.S. Patent No. 6,004,937) or follistatin-domain-containing proteins (e.g., GASP-1 or other proteins as described in U.S. Patent Application Nos. 10/369,736 and 10/369,738); and modulators of metalloprotease activity that affect GDF-8 activation, as described in U.S. Patent Application No. 10/662,438.

[0015] In some embodiments, the GDF-8 inhibitor is a monoclonal antibody or a fragment thereof that blocks GDF-8 binding to its receptor. Nonlimiting illustrative embodiments include a nonhuman monoclonal anti-GDF-8 antibody, e.g., murine monoclonal antibody JA-16 (as described in U.S. Patent Application No. 10/253,532; ATCC Deposit No. PTA-4236);

derivatives thereof, e.g., humanized antibody; and fully human monoclonal anti-GDF-8 antibodies (e.g., Myo29, Myo28, and Myo22, as described in U.S. Patent Application No. 10/688,925; ATCC Deposit Nos. PTA-4741, PTA-4740, and PTA-4739, respectively) or derivatives thereof.

[0016] Corticosteroids, used in the method of the invention include, but are not limited to, beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, triamcinolone acetonide, and derivatives thereof.

[0017] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURES

[0018] Figures 1A and 1B depict results of a histological analysis of diaphragm muscle from *mdx* mice treated for four weeks with anti-GDF-8 neutralizing antibody JA-16 (60 mg/kg, once weekly) and prednisone (2 mg/kg, 3 times a week), prednisone alone, or vehicle control alone. Figure 1A shows severity of muscle fiber atrophy on a 0-4 scale at the end of the trial. Figure 1B shows percentage of affected (atrophied) muscle fibers at the end of the trial. Each bar represents a single mouse.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0019] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0020] The term "**antibody**," as used herein, refers to an immunoglobulin or a part thereof and encompasses any polypeptide comprising an antigen-binding site regardless of the source, method of production, and other characteristics. As a non-limiting example, the term "antibody" includes human, orangutan, mouse, rat, goat, sheep, and chicken antibodies. The term includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, and CDR-grafted antibodies. For the purposes of the present invention, it also includes, unless otherwise stated, antibody fragments such as Fab, F(ab')2, Fv, scFv, Fd, dAb, and other antibody fragments that retain the antigen-binding function.

[0021] Antibodies can be made, for example, via traditional hybridoma techniques (Kohler and Milstein (1975) Nature, 256: 495-499), recombinant DNA methods (U.S. Patent No. 4,816,567), or phage display techniques using antibody libraries (Clackson et al. (1991) Nature, 352: 624-628; Marks et al. (1991) J. Mol. Biol., 222: 581-597). For various other antibody production techniques, see Antibodies: A Laboratory Manual, eds. Harlow et al., Cold Spring Harbor Laboratory, 1988.

[0022] The term "**antigen-binding domain**" refers to the part of an antibody molecule that comprises the area specifically binding to or complementary to a part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen. The epitope or antigenic determinant is a portion of an antigen molecule that is responsible for specific interactions with the antigen-binding domain of an antibody. An antigen-binding domain may be provided by one or more antibody variable domains (e.g., a so-called Fd antibody fragment consisting of a VH domain). An antigen-binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH). .

[0023] The term "**anti-GDF-8 antibody,**" or "**antibody to GDF-8,**" refers to any antibody that specifically binds to at least one epitope of GDF-8. The terms "**GDF-8 receptor antibody**" and "**antibody to a GDF-8 receptor**" refer to any antibody that specifically binds to at least one epitope of a GDF-8 receptor, such as ActRIIB. The term "**neutralizing antibody**" refers to an antibody that is a GDF-8 inhibitor.

[0024] The term "**specific interaction,**" or "**specifically binds,**" or the like, means that two molecules form a complex that is relatively stable under physiologic conditions. The term is also applicable where, e.g., an antigen-binding domain is specific for a particular epitope, which may be present on a number of antigens. Specific binding is characterized by a high affinity and a low to moderate capacity. Nonspecific binding usually has a low affinity with a moderate to high capacity. Typically, the binding is considered specific when

the affinity constant K_a is higher than 10^6 M^{-1}, than 10^7 M^{-1}, or preferably higher than 10^8 M^{-1}. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. Such conditions are known in the art, and a skilled artisan using routine techniques can select appropriate conditions. The conditions are usually defined in terms of concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of non-related molecules (e.g., serum albumin, milk casein), etc.

[0025] The term "**muscle function**" refers to the ability of muscle to perform a physiologic function, such as contraction as measured by the amount of force generated during either twitch or tetanus. Other methods for assessing muscle function are well known in the art and include, but are not limited to, measurements of muscle mass, grip strength, serum CK level, activities of daily living, motion or strength tests, tissue histology (e.g., E&A staining, or collagen III staining), or tissue imaging. Nonlimiting illustrative methods for assessing muscle function are set forth in the Examples.

[0026] The term "**GDF-8**" refers to a specific growth and differentiation factor-8 and, where appropriate, factors that are structurally or functionally related to GDF-8, for example, BMP-11 and other factors belonging to the TGF-β superfamily. The term refers to the full-length unprocessed precursor form of GDF-8 as well as the mature and propeptide forms resulting from post-translational cleavage. The term also refers to any fragments and variants of GDF-8 that maintain at least some biological activities associated

with mature GDF-8, as discussed herein, including sequences that have been modified. The present invention relates to GDF-8 from all vertebrate species, including, but not limited to, human, bovine, chicken, mouse, rat, porcine, ovine, turkey, baboon, and fish (for sequence information, see, e.g., McPherron et al. (1997) Proc. Nat. Acad. Sci. U.S.A., 94: 12457-12461).

[0027] The term "**mature GDF-8**" refers to the protein that is cleaved from the carboxy-terminal domain of the GDF-8 precursor protein. The mature GDF-8 may be present as a monomer, homodimer, or in a GDF-8 latent complex. Depending on conditions, mature GDF-8 may establish equilibrium between any or all of these different forms. In its biologically active form, the mature GDF-8 is also referred to as "**active GDF-8.**"

[0028] The term "**GDF-8 propeptide**" refers to the polypeptide that is cleaved from the amino-terminal domain of the GDF-8 precursor protein. The GDF-8 propeptide is capable of binding to the propeptide binding domain on the mature GDF-8.

[0029] The term "**GDF-8 latent complex**" refers to the complex of proteins formed between the mature GDF-8 homodimer and the GDF-8 propeptide. It is believed that two GDF-8 propeptides associate with two molecules of mature GDF-8 in the homodimer to form an inactive tetrameric complex. The latent complex may include other GDF inhibitors in place of or in addition to one or more of the GDF-8 propeptides.

[0030] The term "**GDF-8 activity**" refers to one or more of physiologically growth-regulatory or morphogenetic activities associated with

active GDF-8 protein. For example, active GDF-8 is a negative regulator of skeletal muscle mass. Active GDF-8 can also modulate the production of muscle-specific enzymes (e.g., creatine kinase), stimulate myoblast proliferation, and modulate preadipocyte differentiation to adipocytes. Exemplary procedures for measuring GDF-8 activity in vivo and in vitro are found in U.S. Patent Application No. 10/688,925, for example.

[0031] As used herein, "**GDF-8 inhibitor**" generally refers to any compound that downregulates the activity of GDF-8, and includes any agent capable of inhibiting activity, expression, processing, or secretion of GDF-8. A GDF-8 inhibitor may, for example, affect stability of or conversion of the precursor molecule to the active, mature form; interfere with the binding of GDF-8 to one or more receptors; or interfere with intracellular signaling of the GDF-8 receptor ActRIIB. Such inhibitors include proteins, antibodies, peptides, peptidomimetics, ribozymes, anti-sense oligonucleotides, double-stranded RNA, and other small molecules, which specifically inhibit GDF-8. Such inhibitors are said to "**inhibit**," "**neutralize**," or "**reduce**" the biological activity of GDF-8.

[0032] The terms "**neutralize**," "**neutralizing**," "**inhibitory**," and their cognates refer to a reduction in the activity of GDF-8 by a GDF-8 inhibitor, relative to the activity of GDF-8 in the absence of the same inhibitor. The reduction in activity is preferably at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or higher. The methods for assessing neutralizing or inhibitory biological activity of GDF-8 inhibitors are known in the art, and can

be performed, for example, using the ActRIIB assay (e.g., as described in Whittemore et al. (2003) BBRC, 300:965-97; or U.S. Patent Application No. 10/253,532) and the RGA assays (as described in Thies (2001) Growth Factors, 18:251-259 or U.S. Patent Application No. 10/253,532).

[0033] The term "**therapeutically effective dose**," or "**therapeutically effective amount**," refers to that amount of a compound that results in prevention, reduction in the risk of occurrence, or amelioration of symptoms in a patient, or a desired biological outcome, e.g., improved muscle function, delayed onset of clinical symptoms, etc. The effective amount can be determined as described in the subsequent sections.

[0034] The terms "**treatment**," "**therapeutic method**," and their cognates refer to treatment or prophylactic/preventative measures. Those in need of treatment may include individuals already having a particular medical disorder as well as those who may ultimately acquire the disorder. Treatment includes any reduction in any symptom of a disorder described in this application. In addition to a reduction or lessening of symptoms, treatment also includes maintaining a patient's current status when worsening is expected, or preventing the occurrence of a symptom in an individual in which the onset of a symptom, disorder, or disease is expected. Treatment may include a decrease or reduction in one or more physiologic function from normal. It may also include a decrease compared to expected symptoms or expected progression of the condition, disorder, or disease.

II. Components for Use in the Methods of the Invention

[0035] In the methods of the present invention, one or more GDF-8 inhibitors are used in combination with one or more corticosteroids.

A. GDF-8 Inhibitors

[0036] GDF-8 inhibitors, used in the methods of the present invention, include, but are not limited to, antibodies to GDF-8; antibodies to GDF-8 receptors; soluble GDF-8 receptors and fragments thereof (e.g., ActRIIB fusion polypeptides as described in U.S. Patent Application No. 10/689,677, including soluble ActRIIB receptors in which ActRIIB is joined to the Fc portion of an immunoglobulin); GDF-8 propeptide and modified forms thereof (e.g., as described in WO 02/068650 or U.S. Patent Application No. 10/071,499, including forms in which GDF-8 propeptide is joined to the Fc portion of an immunoglobulin and/or forms in which GDF-8 is mutated at an aspartate (asp) residue, e.g., asp-99 in murine GDF-8 propeptide and asp-100 in human GDF-8 propeptide); follistatin (e.g., as described in U.S. Patent No. 6,004,937) or follistatin-domain-containing proteins (e.g., GASP-1 or other proteins as described in U.S. Patent Application Nos. 10/369,736 and 10/369,738); and modulators of metalloprotease activity that affect GDF-8 activation, as described in U.S. Patent Application No. 10/662,438.

[0037] In some embodiments, the GDF-8 inhibitor is a monoclonal antibody or a fragment thereof that blocks GDF-8 binding to its receptor. Nonlimiting illustrative embodiments include a nonhuman monoclonal anti-GDF-8 antibody, e.g., murine monoclonal antibody JA-16 (as described in U.S. Patent Application No. 10/253,532; ATCC Deposit No. PTA-4236);

derivatives thereof, e.g., humanized antibodies; and fully human monoclonal anti-GDF-8 antibodies (e.g., Myo29, Myo28, and Myo22, as described in U.S. Patent Application No. 10/688,925; ATCC Deposit Nos. PTA-4741, PTA-4740, and PTA-4739, respectively), or derivatives thereof.

[0038] In some embodiments, the GDF-8 inhibitor blocks GDF-8 from binding to its receptor, by binding to GDF-8 or to the GDF-8 receptor. In various embodiments, the GDF-8 inhibitor is an anti-GDF-8 antibody that has the affinity to GDF-8, expressed as an affinity constant (K_a), wherein K_a is at least 10^5 M^{-1}, 10^6 M^{-1}, 10^7 M^{-1}, 10^8 M^{-1}, 10^9 M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1}, or 10^{12} M^{-1}. Also contemplated for use in humans are inhibitors that are humanized forms and derivatives of nonhuman antibodies derived from any vertebrate species described in patent applications cited herein, or in Antibody Engineering, ed. Borrebaeck, 2nd ed., Oxford University Press, 1995; and Antibodies: A Laboratory Manual, eds. Harlow et al., Cold Spring Harbor Laboratory, 1988.

B. Corticosteroids

[0039] Corticosteroids used in the methods of the present invention, include, but are not limited to, beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, prednisone, mometasone furoate, triamcinolone acetonide, and derivatives thereof.

[0040] Pharmaceutically acceptable salts of compounds disclosed herein can also be used.

[0041] Corticosteroids are available commercially in various pharmaceutical formulations (Physician's Desk Reference (PDR) 2003, 57th ed., Medical Economics Company, 2002). For example, oral formulations are

commercially available for cortisone, hydrocortisone (Cortef®), prednisone (Deltasone®, Meticorten®, Orasone®), prednisolone (Delta-Cortef®, Pediapred®, Prelone®), triamcinolone (Aristocort®, Kenacort®), methylprednisolone (Medrol®), dexamethasone (Decadron®, Dexone®, Hexadrol®), betamethasone (Celestone®), and deflazacort (Calcort®). Other formulations of these and other corticosteroids can be used in the methods of the invention.

C. Therapeutic and Prophylactic Methods

[0042] The invention provides method of treating mammalian subjects, including methods to treat loss of muscle function, muscle weakness, and/or corticosteroid-induced muscle atrophy.

[0043] Methods of the invention comprise administering to the mammal a therapeutically effective amount of at least one GDF-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat at least one of loss of muscle function, muscle mass, muscle weakness, muscle atrophy, or cardiomyopathy. The methods can be used for treating neuromuscular disorders such as muscular dystrophies. In some embodiments, muscle function is improved relative to the same treatment either in the absence of the GDF-8 inhibitor or the corticosteroid. The muscles treated include, but are not limited to, gastrocnemius, tibialis, anterior, quadriceps, extensor digitorum, cardiac muscle, or diaphragm muscle.

[0044] Neuromuscular disorders include, but are not limited to, any acute or chronic disease or disorder that compromises muscle function,

causes muscular injury, or otherwise causes a diminution in muscle mass and/or function. A wide variety of diseases or disorders is known and includes, for example, muscular dystrophies such Duchenne muscular dystrophy, Becker muscular dystrophy, Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, rigid spine syndrome, Ullrich syndrome, Fukuyama muscular dystrophy, Walker-Warburg syndrome, muscle-eye-brain disease, facioscapulohumeral muscular dystrophy, congenital muscular dystrophy, myotonic dystrophy (Steinert's disease), nondystrophic myotonia, periodic paralyses spinal muscular atrophy, familial amytrophic lateral sclerosis, hereditary motor and sensory neuropathy, Charcot-Marie-Tooth disease, chronic inflammatory neuropathy, distal myopathy, myotubular/centronuclear myopathy, nemaline myopathy, mini core disease, central core disease, desminopathy, inclusion body myositis, mitochondrial myopathy, congenital myasthenic syndrome, post-polio muscle dysfunction, and disorders described in Emery (2002) The Lancet, 359:687-695; and Khurana et al. (2003) Nat. Rev. Drug Disc., 2:379-386. Patients may exhibit mild, moderate or severe muscle weakness, muscle wasting, and effects on independent ambulation associated with such a disorder. Patients having or at risk for developing these disorder will benefit from GDF-8 inhibitor and a corticosteroid.

[0045] In general, a patient who will benefit from coadministration of a GDF-8 inhibitor and a corticosteroid is one who exhibits a 2-10-fold or higher increase in the serum CK activity, a positive family history, an abnormal

variation in the diameter of muscle fibers, a deficiency in dystrophin or a mutation in the dystrophin gene, loss of muscle mass, muscle weakness, cardiomyopathy, and/or loss of muscle strength. The diagnostic procedures, including the appropriate genetic testing, are described in Diagnostic Criteria for Neuromuscular Disorders, ed. Emery, 2nd ed., Royal Society of Medicine Press, 1997. The combination treatment can be also beneficial to subjects undergoing corticosteroid therapy for disorders other than neuromuscular disorders and/or subjects with a history of a long-term corticosteroid use so long these subjects exhibit, or are at risk of diminution of muscle function such as characterized by muscle weakness, loss of muscle mass, and/or muscle atrophy, etc. Examples of disorders for which corticosteroid therapy is often used include, but are nor limited to, asthma, allergy, arthritis, dermatologic disorders (e.g., inflammatory dermatoses, eczema, psoriasis, etc), lupus erythematosus, and other chronic inflammatory conditions.

[0046] Methods of administration and compositions used in the methods of the inventions are provided. Administration is not limited to any particular delivery system and may include, without limitation, parenteral (including subcutaneous, intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection) rectal, topical, transdermal, or oral (for example, in capsules, suspensions, or tablets). Administration to an individual may occur in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier and/or additive as part of a pharmaceutical

composition. Physiologically acceptable salt forms and standard pharmaceutical formulation techniques and excipients are well known to persons skilled in the art (e.g., as described in Physician's Desk Reference (PDR) 2003, 57th ed., Medical Economics Company, 2002; and Remington: The Science and Practice of Pharmacy, eds. Gennado et al., 20th ed, Lippincott, Williams & Wilkins, 2000).

[0047] A GDF-8 inhibitor and a corticosteroid are administered concurrently or consecutively over overlapping or nonoverlapping intervals. In the sequential administration, the GDF-8 inhibitor and the corticosteroid can be administered in any order. In some embodiments, the length of an overlapping or nonoverlapping interval is more than 2, 4, 6, 12, 24, or 48 weeks.

[0048] For corticosteroids, the prescribing physician routinely selects the dosage and regimen. For example, prednisone is used at about 0.1-2 mg per kilogram of body weight per day, and most commonly at 0.5-1 mg/kg/day, e.g., 0.75 mg/kg/day. The corticosteroid may be administered at average weekly doses of approximately 1-14 mg/kg body weight, including approximately 1, 2, 5, 7, 10, 12, or 15 mg/kg body weight per week, and the prescribing physician may select a frequency of administration as appropriate. Single dose, continuous or periodic corticosteroid administration may be selected, including administration at hourly, daily, bi-weekly, weekly, or other periodic intervals. Preferably, corticosteroids are administered orally or by injection 1-4 times per day. Corticosteroid dosage may be optimized as a

combination therapy, and dosage may be lowered to reduce significant side effects of administration.

[0049] The GDF-8 inhibitors can be administered alone or in a mixture with a corticosteroid or another compound. GDF-8 inhibitors can be administered at a dose of approximately from 1 µg/kg to 25 mg/kg, depending on physiology, the severity of the symptoms and the progression of the disease. Single dose, continuous, or periodic administration may be selected, with intervals between GDF-8 inhibitor doses chosen from hourly, daily, bi-weekly, weekly, bi-monthly, monthly, or other appropriate intervals. For example, GDF-8 inhibitors such as antibodies may be administered in an outpatient setting by weekly administration at about 0.1-10 mg/kg dose by intravenous (IV) infusion, intraperitoneal, or subcutaneous injection. In general, the appropriate therapeutically effective dose of a GDF-8 inhibitor is selected by a treating clinician and would range approximately from 1 µg/kg to 20 mg/kg, from 1 µg/kg to 10 mg/kg, from 1 µg/kg to 1 mg/kg, from 10 µg/kg to 1 mg/kg, from 10 µg/kg to 100 µg/kg, from 100 µg to 1 mg/kg, and from 500 µg/kg to 5 mg/kg. Exemplary effective doses of GDF-8 inhibitor include approximately 0.1, 0.3, 0.5, 1, 5, 10, or 20 mg/kg/wk. Additionally, specific dosages indicated in the Examples or in the Physician's Desk Reference (PDR) 2003, 57th ed., Medical Economics Company, 2002, can be used.

D. Methods of Testing Compounds for Therapeutic Efficacy

[0050] The invention further provides methods for testing in an animal, e.g., a rodent or a primate, whether a therapeutic compound is efficacious when administered in combination with at least one GDF-8 inhibitor and at

least one corticosteroid. In some embodiments, the method of evaluating the efficacy of a compound comprises: administering the compound to a first animal in combination with a GDF-8 inhibitor and a corticosteroid; administering the GDF-8 inhibitor and the corticosteroid to a second animal; determining the level of muscle function in the first and in the second animal after the administrations; and comparing the levels of muscle function. If the level in the first animal is lower than the level in the second animal, it indicates that the compound or the combination is efficacious.

[0051] In other embodiments, the compound may be evaluated for efficacy in treatment of muscular dystrophy when administered in combination with a GDF-8 inhibitor and/or a corticosteroid.

[0052] Several animal models are available for such evaluative purposes. For example, the *mdx* model has been described, for example, by Torres et al. (1987) Brain, 110:269-299, and Hoffman et al. (1987) Science, 238:347-350. Extremely high levels of CK are consistently noted with dystrophin-deficiency in *mdx* mice and DMD humans due to sarcolemmal damage (Bulfield et al. (1984) Proc. Natl. Acad. Sci. USA, 81:1189-1192; and Matsuda et al. (1995) J. Biochem. (Tokyo), 118: 959-64). As another example, two other animal models can be used: *utr-/- mdx* mice (Gillis (2002) Neuromuscul. Disord., 12(1):90-84; and Deconick et al. (1997) Cell, 90:729-738) and *nu-/- mdx* mice (Morrison et al. (2000) Lab. Invest., 80:881-891).

EXAMPLES

Example 1: Effect of GDF-8 neutralizing antibody on dystrophic muscle

[0053] The ability of *in vivo* inhibition of GDF-8 to ameliorate muscular dystrophy was tested in the *mdx* mouse model of DMD. Five to seven week old male C57BL/10ScSn-*mdx*/j mice (Jackson Laboratory, Bar Harbor, ME) were treated with weekly intraperitoneal injections of the GDF-8 neutralizing murine antibody JA-16 (60 mg/kg, double dosing at first week, n=11), and vehicle alone (control group, n=10) for 12 weeks. These mice were also compared to mice of the same background strain (C57BL/10, n=12) without the dystrophin deficiency.

[0054] The body weight was monitored before, during and after treatment. Mice in the treatment group gained weight relative to mice in the vehicle control group. Results are shown in Table 1.

Table 1. Total body weight (g) Average values with SEM

Week of trial	JA-16 (*mdx*)	vehicle control (*mdx*)	vehicle control (non-*mdx*)
0	21.92+/-0.42	22.51+/-0.36	19.18+/-0.40
4	27.82+/-0.43	26.76+/-0.60	24.14+/-0.27
8	29.59+/-0.54	28.49+/-0.58	25.31+/-0.28
12	32.42+/-0.57	31.12+/-0.73	27.17+/-0.39

[0055] Mice were also subjected to a grip test after 6 and 10 weeks of dosing. Mice in the treatment group at four and ten weeks had 9% ($p = 0.09$) and 19% ($p < 0.05$) respectively greater grip strength than mice in the vehicle control groups. Results are shown in Table 2.

Table 2. Grip strength (lb) Average values with SEM

Week of trial	JA-16 (*mdx*)	vehicle control (*mdx*)	vehicle control (non-*mdx*)
6	0.261+/-0.011	0.239+/-0.006	0.239+/-0.011
10	0.249+/-0.006	0.210+/-0.014	0.247+/-0.010

[0056] To quantify the difference in muscle mass between treatment and vehicle control, animals were sacrificed and quadriceps and gastrocnemius muscles dissected out and weighed. Quadriceps muscles from the treated group of animals weighed 13% more than controls (0.371±0.009 vs. 0.317±0.008 g; $p<0.05$). Gastrocnemius muscles from the treated group of animals weighed 17% more than controls (0.223±0.008 vs. 0.197±0.005 g; $p<0.0005$).

Example 2: Effect of GDF-8 neutralizing antibody and prednisone on normal and dystrophic muscle

[0057] Male C57BL/10ScSn-*mdx*/j and C57BL/10 (Jackson Laboratory, Bar Harbor, ME). Mouse monoclonal anti-GDF-8 antibody JA-16, prednisone (P-9901, Sigma), or vehicle (peanut oil) was injected starting at age 5-7 weeks for 4 weeks. Mice were intraperitoneally (IP) injected with JA-16 at a dose of 60 mg/kg per week (double dosing at first week), or subcutaneously (SC) injected with prednisone at 2 mg/kg, 3 times a week.

[0058] The body weight and grip strength were monitored before, during and after treatment. Results are shown in Table 3 and Table 4, respectively.

Table 3. Total body weight (average ± SEM, g)

Week of trial	Prednisone + JA-16 (*mdx*)	Prednisone (*mdx*)	vehicle control (*mdx*)	vehicle control (non-*mdx*)
0	17.7±1.6	17.6±1.8	16.0±1.9	19.2±0.4
1	22.1±1.4	20.9±1.8	19.1±2.1	22.4±0.3
2	25.9±1.2	24.2±1.6	23.7±1.4	23.8±0.4
3	26.5±1.1	24.7±1.5	24.8±1.2	24.9±0.4
4	28.1±1.2	25.9±1.6	25.7±1.4	25.6±0.5

Table 4. Grip strength (average ± SEM, lb)

Week of trial	Prednisone + JA-16 (*mdx*)	Prednisone (*mdx*)	vehicle control (*mdx*)	vehicle control (non-*mdx*)
0	0.161±0.018	0.144±0.010	0.164±0.014	0.164±0.009
3	0.219±0.019	0.177±0.006	0.168±0.005	0.212±0.010
4	0.281±0.011	0.213±0.011	0.217±0.010	0.234±0.018

[0059] At the end of the study, mice were sacrificed and muscle mass was assessed by dissecting and weighing the gastrocnemius and quadriceps. Results are shown in Table 5. To confirm biological activity of prednisone, sera from a separate cohort of mice were collected and analyzed for IL6 and IL1β (Ani Lytics, Inc., Gaithersburg, MD). Both cytokines were found to be reduced in the sera of mice treated with prednisone.

Table 5. Muscle weight (average ± SEM, g)

Muscle	Prednisone + JA-16 (*mdx*)	Prednisone (*mdx*)	vehicle control (*mdx*)	vehicle control (non-*mdx*)
Gastroc	0.364±0.019	0.287±0.023	0.299±0.019	0.280±0.010
Quad	0.527±0.030	0.417±0.029	0.415±0.030	0.392±0.010

[0060] Therefore, the results demonstrate that in muscular dystrophy, administration of an inhibitor of GDF-8, i.e., anti-GDF-8 antibody, and

prednisone is effective in increasing muscle mass and strength relative to treatment with prednisone alone or vehicle.

[0061] Furthermore, in these studies the effects of JA16 plus prednisone treatment (Example 2) were greater than the effects of treatment with JA16 alone (Example 1). The increase in body weight compared to vehicle after four weeks of treatment was more dramatic for JA16 plus prednisone treatment than for JA16 treatment alone. The increase in grip strength compared to vehicle control after four weeks of treatment with JA16 plus prednisone was greater that the increase after six or ten weeks of treatment with JA16 alone. The increase over vehicle control in muscle mass after four weeks of treatment with JA16 plus prednisone was also greater than the increase after twelve weeks of treatment with JA16 alone.

Example 3: Effect of GDF-8 neutralizing antibody on prednisone-induced muscle atrophy

[0062] In the mice treated as described in Example 2,diaphragm muscle was histologically examined as described in Example 1. The morphological changes were evaluated by an independent pathology lab that had no knowledge of the treatment group assignments. Severity grades were assigned on a scale from 0 to 4 (0 = none; 1 = minimal; 2 = mild; 3 = moderate; and 4 = marked). Results are shown in Figure 1A (severity scores) and Figure 1B (percentage of muscle fibers atrophied). The results show that administration of the anti-GDF-8 antibody with prednisone reduces prednisone-induced muscle atrophy.

Example 4: Treatment of Muscular Dystrophies

[0063] As an example of treating MD in humans, the Myo29 antibody is administered in combination with prednisone or prednisolone. Nonlimiting exemplary treatment regimens and outcomes are summarized in Table 6. Other treatment regimens can be determined by a treating physician, with ranges of the corticosteroids and GDF-8 inhibitors dosage and administration as discussed above.

Table 6

Patient No.	Treatment Regimen	Treatment Goal
Patient 1	Myo29 at 10 mg/kg/week, administered by bi-weekly injection plus prednisone at 0.75 mg/kg/day for 2 years, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength, and function over benefit of prednisone alone
Patient 2	Myo29 at 0.1 mg/kg/week, administered by weekly IV plus prednisone at 1.0 mg/kg/day, continuing treatment as needed.	Maintenance and/or increase of muscle mass, strength, and function over benefit of prednisone alone
Patient 3	Myo29 at 1 mg/kg/week administered by monthly injection plus prednisone at 0.50 mg/kg/day for 2 years, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function or increased preservation of function for muscle groups that are not already compromised over benefit of prednisone alone
Patient 4	Myo29 at 20 mg/kg/week, administered in a single dose by IV plus prednisone at 0.75 mg/kg/day for 2 years, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function or increased preservation of function over benefit of prednisone alone
Patient 5	Myo29 at .1 mg/kg/week, administered in a single dose by IV plus prednisone at 5 mg/kg/wk, as needed	Maintenance and/or increase of muscle mass, strength and function over benefit of prednisone alone
Patient 6	Myo29 at 1 mg/kg/week, administered weekly by IV plus prednisone at 2 mg/kg/wk for at least 2 months, or as needed	Maintenance and/or increase of muscle mass, strength and function over benefit of prednisone alone

Patient 7	Myo29 at 10 mg/kg/week, administered in a single dose by subcutaneous injection plus prednisone at 7 mg/kg/wk for at least 6 months, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function over benefit of prednisone alone
Patient 8	Myo29 at 20 mg/kg/week, administered weekly by injection plus prednisone at 14 mg/kg/wk for 2 years, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function over benefit of prednisone alone
Patient 9	Myo29 at 1 mg/kg/week, administered bi-monthly by IV plus prednisone at 10 mg/kg/wk for at least 1 month, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function over benefit of prednisone alone
Patient 10	Myo29 at 0.3 mg/kg/week, administered monthly by subcutaneous injection plus prednisone at 0.75 mg/kg/day for 1 year, or continuing treatment as needed	Maintenance and/or increase of muscle mass, strength and function or increased preservation of function for muscle groups over benefit of prednisone alone

[0064] All publications and patents cited and sequences identified by accession or database reference numbers in this disclosure are incorporated by reference in their entirety.

CLAIMS

1. A method of treating a mammal with a decrease of muscle function, comprising administering to the mammal a therapeutically effective amount of at least one GDF-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat decrease of muscle function.

2. The method of claim 1, wherein the muscle function of at least one muscle is evaluated by at least one parameter chosen from muscle mass, muscle contraction force, serum CK concentration, or muscle morphology.

3. The method of claim 1, wherein the muscle whose function is treated is chosen from at least one of gastrocnemius, tibialis anterior, quadriceps, extensor digitorum longus, cardiac muscle, or diaphragm muscle.

4. The method of claim 1, wherein treating said mammal results in increased body weight of said mammal.

5. The method of claim 1, wherein treating said mammal results in increased grip strength.

6. A method of treating muscle weakness, comprising administering to a mammal a therapeutically effective amount of at least one GDF-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat loss of muscle strength.

7. A method of treating corticosteroid-induced muscle atrophy, comprising administering to a mammal a therapeutically effective amount of at least one GDF-8 inhibitor sufficient to treat the corticosteroid-induced muscle atrophy.

8. A method of treating a neuromuscular disorder, comprising administering to a mammal having or at risk of the neuromuscular disorder a therapeutically effective amount of at least one GDF-8 inhibitor and a therapeutically effective amount of at least one corticosteroid in the amounts and for a period of time sufficient to treat the neuromuscular disorder.

9. The method of claim 8, wherein the neuromuscular disorder is a muscular dystrophy.

10. The method of claim 9, wherein the muscular dystrophy is Duchenne muscular dystrophy.

11. The method of claim 9, wherein the muscular dystrophy is Becker muscular dystrophy.

12. The method as in any one of claims 1-11, wherein the mammal is human.

13. The method as in any one of claims 1-11, wherein the corticosteroid is chosen from at least one of:

(a) at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide;

(b) a derivative of at least one of beclomethasone dipropionate, budesonide, cortisol, dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetonide; or

(c) a pharmaceutically acceptable salt of at least one of beclomethasone dipropionate, budesonide, cortisol,

dexamethasone, fluticason propionate, mometasone furoate, prednisone, or triamcinolone acetoniden.

14. The method as in any one of claims 1-11, wherein the corticosteroid is prednisone or prednisolone.

15. The method as in any one of claims 1-11, wherein the corticosteroid is administered at a dose between 0.1 and 2.0 mg/kg/day.

16. The method as in any one of claims 1-11, wherein the corticosteroid is administered orally.

17. The method as in any one of claims 1-11, wherein the GDF-8 inhibitor is chosen from an antibody to GDF-8, an antibody to a GDF-8 receptor, a soluble GDF-8 receptor, a GDF-8 propeptide, a small molecule inhibitor of GDF-8, follistatin, or a follistatin-domain-containing protein.

18. The method of claim 17, wherein the antibody to GDF-8 is chosen from JA-16, Myo29, Myo28, or Myo22.

19. The method of claim 17, wherein the GDF-8 propeptide is mutated at an aspartate residue.

20. The method of claim 17, wherein the GDF-8 propeptide is joined to the Fc portion of an immunoglobulin.

21. The method of claim 17, wherein the GDF-8 receptor is ActRIIB.

22. The method of claim 17, wherein the GDF-8 receptor is joined to the Fc portion of an immunoglobulin.

23. The method of claim 17, wherein the GDF-8 inhibitor is follistatin.

24. The method of claim 17, wherein the follistatin-domain-containing protein is GASP-1.

25. The method of claim 17, wherein the GDF-8 inhibitor is a small molecule inhibitor.

25. The method of claim 1, wherein the method results in treating of cardiomyopathy of said mammal.

26. The method of claim 1, wherein the administration of GDF-8 inhibitor and corticosteroid is concurrent.

27. The method of claim 1, wherein the administration of GDF-8 inhibitor and corticosteroid is consecutive.

28. The method of claim 8, wherein the administration of GDF-8 inhibitor and corticosteroid is concurrent.

29. The method of claim 8, wherein the administration of GDF-8 inhibitor and corticosteroid is consecutive.

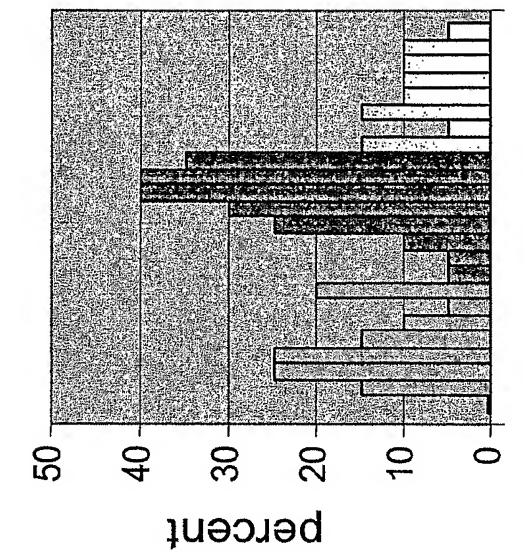
percent
50
40
30
20
10
0
Prednisone + JA-16
Prednisone
Vehicle

FIG. 1B

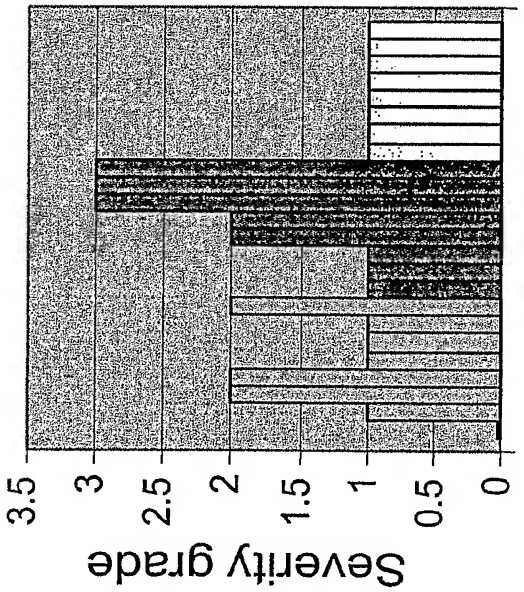
Severity grade
3.5
3
2.5
2
1.5
1
0.5
0
Prednisone + JA-16
Prednisone
Vehicle

FIG. 1A